Device for selectively controlling the temperature of individual containers

Description

The present patent application relates to a device for selectively controlling the temperature of individual containers, especially for application in the field of molecular biology and in screening, more especially in high-throughput-screening (HTS).

Pharmaceutical research has been revolutionised by the opportunities afforded by combinatorial chemistry, because those methods enable a large number of different substances having pharmacological potential to be produced very efficiently. The starting point for pharmaceutical research nowadays is a substance library which holds a large number of chemical compounds, for example natural substances, but also synthetic peptides as well as nucleic acids. In so-called high-throughput-screening, very large numbers of such substances from the substance library are investigated for their pharmacological activity using biological test procedures in a partially automated process. For that purpose, *inter alia* molecular-biological techniques are also employed. The entire process is generally carried out using pipetting robots and handling robots. More recent techniques using miniaturised systems in this field are described in WO 98/00231. Special containers for HTS are described in DE 298 05 613 and WO 97/15394.

The containers in which the substances of the substance library are stored are generally container arrays, for example in 96-, 384- or 1536-microtitration plate format. A container array in 96-microtitration plate format is described, for example, in US 4,154,795. Various systems are available, *inter alia* so-called cluster racks which are array devices for accommodating a plurality of test tubes. Other formats are also possible, however, such as, for example, conical reaction vessels, such as are used, for example, for the amplification of nucleic acids. As a rule, such containers are closed by sealing. More recent developments in this respect indicate possible ways of closing such containers by means of individual lids. For example, DE 44 12 286 describes lids amenable to automation which can

also be used in the field of HTS.

The substance libraries usually also contain very temperature-sensitive substances which have to be stored at low temperatures, for example at -20°C or -70°C. Repeated thawing and refreezing damages such substances and must therefore be avoided.

There are various strategies for the use of the substance library in HTS:

1. A plurality of "daughter plates" are pipetted from a so-called "mother plate". The "daughter plates" are then supplied to the appropriate biological assay in HTS.

2. Only predetermined individual compounds, that is to say individual containers, of the substance library are used to compile a range of substances in a "daughter plate" which is then supplied to the HTS process.

The last-mentioned process, especially, makes it necessary for individual test tubes or containers of an array to be thawed, to have some of the substances removed and to be frozen again after closure.

In order that all the containers of a plate do not have to be thawed and refrozen unnecessarily often, the prior art already discloses devices by means of which it is possible selectively to control the temperature of (that is to say, for example, to thaw) individual reaction vessels of an array, while the remaining containers remain frozen. Such devices accordingly enable the substances in a container array, some of which are valuable, to be kept for a relatively long time and render the substance libraries usable over a relatively long period.

For example, WO 99/16549, which has an earlier priority but was post-published, discloses a holding plate for a container array of 96 individual containers. Each of the individual holders of the holding plate is equipped with its own heating device

which enables the temperature of the individual container housed in that holder to be controlled, especially thawed. The known temperature-control device therefore has an excessively elaborate construction and is therefore correspondingly expensive to manufacture and to operate. It can easily be seen that the expense involved here for 96 individual containers assumes uneconomical proportions when microtitration plates in 384- or 1536-format are used.

DE 1 900 279 discloses a temperature-control device having a plurality of temperature-control elements which can together be brought into heat-exchange contact with a corresponding plurality of containers of a container array. The temperature-control elements can for that purpose be displaced in the vertical direction, while the container array can be displaced relative to the temperature-control elements in the horizontal plane. A disadvantage, however, is that the heat emanating from the temperature-control elements can dissipate unimpeded in the container array and accordingly there is a risk that selective thawing of a row of containers will undesirably result in at least the immediately adjacent rows of containers also being thawed.

Reference is also made to DE 34 41 179, but the temperature-control device for microtitration plates described therein does not contain a selective thawing unit. Here it is rather a matter of maintaining the temperature of the reaction vessels of the array at as uniform a level as possible. The same is true of DE 39 41 168 and DE 39 38 565.

Finally, as regards the further prior art, reference is also made to DE 1 573 193, DE 33 07 572, DE 40 22 792, DE 196 46 115, DD 239 473, DD 276 547, US 4,116,777, US 4,950,608, US 5,849,208 and WO 98/15969.

Accordingly, the aim of the invention is to provide a device which, while having a simple and economically producible structure, makes it possible selectively to control the temperature of individual containers of a container array and which at the same time ensures that adjacent containers, the temperature of which is not to be controlled, are not appreciably affected as a result and, in particular, ensures

that, when individual containers are thawed, the other containers of the container array are not likewise at risk of being thawed.

That aim is achieved according to the invention by a device having the features of claim 1. In such a device, the containers of the container array the temperature of which is not to be controlled can, by means of the holding plate, be maintained at a temperature which ensures that they remain frozen. On the other hand, the containers the temperature of which is to be controlled by the heat-exchange contact with the temperature-control element are arranged in the region of an through-opening in the holding plate and are accordingly not cooled thereby. The relative movements of container array and temperature-control element(s) can be brought about in a simple manner by a robot. Such robots are anyway present in modern systems in the form of pipetting robots or handling robots, for example for carrying out molecular-biological investigations on samples held in the containers of a container array, and are therefore not a factor in determining the cost of the device according to the invention.

A particular aspect of the present invention is the provision of at least one additional cooling zone which dissipates the heat emitted by the temperature-control element and not utilised for controlling the temperature of the container. That additional cooling zone provides an additional substantial safeguard against the undesirable effect of heat on containers of the container array the temperature of which is not to be controlled. Independent protection is therefore sought for that aspect also.

A further use for a device according to the invention for selectively controlling the temperature of individual containers of a container array is the hybridisation of single-strand nucleic acids. In order to achieve the highest degree of stringency in the hybridisation there is used *inter alia* a process which makes the temperature control of the reaction container conditional on a special hybridisation temperature that is specific to each sequence. If a plurality of containers with different nucleic acid sequences are to be hybridised in one working step, a device according to the invention is of considerable advantage because a plurality of vessels can then

be maintained at different hybridisation temperatures in parallel.

The invention is described in greater detail below using exemplary embodiments with reference to the accompanying drawing.

Fig. 1 shows a roughly diagrammatic perspective view of a temperature-control device according to the invention;

Fig. 2 is a plan view of the device according to Fig. 1;

Fig. 3 is a detailed view of a temperature-control rod;

Fig. 4 is a rough diagrammatic side view of the temperature-control device according to Figures 1 and 2;

Fig. 5a, 5b show views of an array plate of a plurality of individual containers, Fig. 5a being a perspective view from below and Fig. 5b being a side view in section showing dimensions (in mm); and

Fig. 6 – 8 show roughly diagrammatic views of further embodiments of temperature-control devices according to the invention.

The arrangement according to the invention is based on two elements: firstly the special containers for storing and optionally treating the substances of the substance library and secondly the device for selective temperature control.

Fig. 5a shows a container array 30 in 96-microtitration plate format. The container array 30 comprises a plate 11, on the underside of which a plurality of individual containers 3 is integrally formed, for example in an injection-moulding process. It is characteristic that the individual reaction containers 3 are arranged freely and without connecting ribs underneath the array plate 11. On the upper side of the array plate 11 there are provided the openings 13 of the containers 3 (see Fig. 5b). These can be closed by means of individual lids, such as are known, for example, from DE 44 12 286. Other possible methods of closure will also be known to the person skilled in the art, however.

In addition to the arrangement shown in Fig. 5a and 5b, however, other embodiments are also possible. For example, the individual containers 3 need not

necessarily be combined to form a permanent array 30. It is alternatively equally possible for a plurality of separately constructed "loose" containers to be combined to form an array at least occasionally or temporarily using a special perforated plate provided for that purpose (also referred to as a "rack" in technical language).

According to the invention it is merely necessary that the lower portions of the reaction containers 3 are freely accessible. For example, the spacing A between the individual reaction containers 3 can be between about 1 mm and about 10 mm, preferably between about 2 mm and about 5 mm. In accordance with the view showing dimensions in Fig. 5b, the spacing A has a value of 3.25 mm.

Furthermore, the distance B from the edge of the openings 13 to the base of the container 3 can be between about 1 mm and about 100 mm, preferably between about 5 mm and about 35 mm. In accordance with Fig. 5b, the spacing B has a value of 22.6 mm. In the embodiment according to Fig. 5, the length of the zone 14 freely hanging down below the plate 11 (see Fig. 1) is 18 mm.

The shape of the containers 3 tapers at least over a portion of the length from the opening 13 to the base, preferably conically so that sufficient space is available for the positioning of a temperature-control or thawing rod 2 (see, for example, Fig. 2). For rapid thawing or freezing it is also advantageous for the individual containers in the free-standing zones 14 to be thin-walled below the array plate 11 in order to promote rapid heat-exchange. Wall thicknesses of between about 0.2 mm and about 0.5 mm, preferably between about 0.2 mm and about 0.3 mm, are advantageous.

In order to keep the exchange of heat *via* the plate 11 as low as possible, the plate should be as thin as possible. This detracts, however, from the stability of the container array 30. The thickness of the array plate 11 should therefore be between about 0.5 mm and about 5 mm, preferably between about 2 mm and about 4 mm. In accordance with Fig. 5b, the plate 11 has a thickness of about 4 mm.

The container arrays 3 can be made, for example, of thermally deformable plastics, such as polyethylene, polypropylene, polystyrene, polycarbonate, polyurethane or the like. They can be produced simply and economically using injection-moulding methods.

For selectively controlling the temperature of one or more individual containers 3 of the container array 30, according to the invention the temperature-control device 40 shown in detail in Figures 1 to 4 is used. According to Fig. 1, the device 40 comprises a holding plate 1 which is preferably made of a material having good thermal conductivity, for example metal, preferably aluminium, brass or copper. The plate 1 can be cooled with the aid of cooling devices familiar to the person skilled in the art and known *per se*, for example Peltier elements, compressor cooling units in conjunction with suitable heat transmission elements or the like. The purpose of such cooling is to keep the substances held in the container array 30 in the frozen state after removal from the freezer.

In the plate 1 there are depressions 4 which serve as holders for the containers 3 and the form of which is therefore selected to correspond to the outer contours of the containers 3. In the embodiment shown, the depressions 4 are in the form of longitudinal channels, so that the containers 3 can be displaced therein in the direction of the double-headed arrow y, that is to say in the Y-direction. Approximately in the centre of the displacement path of the plate 1, each longitudinal channel is provided with at least one through-opening 5. A temperature-control element, for example in the form of a temperature-control or thawing rod 2, can be introduced into each of the through-openings 5.

The object of that temperature-control or thawing rod 2 is to control the temperature of, or thaw, the container 3 arranged in the region of the respective through-opening 5. For that purpose, by a vertical movement upwards in the direction of the arrow z, that is to say in the Z-direction, the temperature-control element 2 can be brought into physical heat-exchange contact with the surface of the respective container 3. For using the temperature-control device according to

the invention for multiple hybridisation at different temperatures, a plurality of temperature-control elements 2 are introduced into a plurality of through-openings 5.

According to Fig. 2, the through-openings 5 are surrounded by cooling zones 16 of which only one is shown in Fig. 2. Those cooling zones serve to dissipate as effectively as possible the heat emitted by the temperature-control or thawing rod 2 that is not utilised for controlling the temperature of, or thawing, the container 3, in order that any undesirable effect of that heat on adjacent containers 3 can thus be prevented. The cooling zones 16 can comprise, for example, ring elements made of a material having good thermal conductivity which are thermally coupled to an external cooling device. The ring elements can be solid rings made, for example, of copper or the like, or they may be ring-shaped pipes through which a cooling fluid flows. When a plurality of such additional cooling zones is provided, at least some of those cooling zones can be thermally coupled to a common cooling device. The temperature difference between the cooling zones 16 and the coolable holder 1 is advantageously between about 5°C and about 50°C, preferably between about 20°C and about 30°C.

In the embodiment according to Fig. 2, each of the longitudinal channels 4 has a single through-opening 5. Furthermore, a linear movement unit 7 having a movable rake 6 is provided, by means of which the container array 30 held in the plate 1 can be moved in the Y-direction. In that way it is ensured that each container 3 of the container array 30 can be moved over a temperature-control or thawing position 5. In addition, the temperature-control or thawing rod 2 is displaceable in the X-direction. By movement of the container array 30 in the Y-direction as well as of the rod 2 first in the X-direction and then in the Z-direction, it is thus possible to move to and thus control the temperature of, or thaw, any desired container 3 of the container array 30 .

In principle, however, it is also possible for the holding plate 1 to have a number of holders corresponding to the number of containers 3 of the container array 30, each of which is provided with an through-opening 5, and for the temperature-

control or thawing element 2 to be movable in all three spatial directions, that is to say both in the X and Y directions and in the Z-direction.

Fig. 3 shows the structure of the temperature-control or thawing element 2 used according to the invention. It comprises an insulating layer 8, which ensures that the transmission of heat between the temperature-control or thawing rod 2 and the surrounding area, for example the metal plate 1, is minimal. As a result, the radiation or emission of heat that is not utilised for controlling the temperature of the vessel 3 in question is kept at a low level. The interior of the temperature-control or thawing rod 2 is preferably made of a ceramic material 8 having an electrically heatable coil 9. In principle, it would also be possible to use microwave or infrared heating means. Heating coils embedded in a suitable material, such as ceramics, have the advantage, however, that their use for reaction mixtures containing biologically active substances does not damage those substances by radiation effects or the like.

Fig. 4 shows a further detail according to the invention in a special embodiment: when the temperature-control or thawing rod 2 travels upwards, it must be pressed against the container to be thawed in order to achieve good transition of heat. There is a risk, however, that the container array 30 will be lifted out of the coolable holder 1 as a result. In order to avoid this, when the temperature-control element 2 is being moved upwards, a counterhold device, for example a counterhold plate 15, is automatically moved downwards and presses the container array 30 into the holding channels 4 of the coolable plate 1. When a device is used for automatically removing or attaching a lid associated with the individual container to be temperature-controlled, the counterhold device can also be formed by a gripper arm of that lid-handling device. This further simplifies the construction of the device according to the invention, because there is no need to provide a separate counterhold device.

Surprisingly it has been found that by virtue of the structure described above, very rapid heating of the sample containers 3 is possible without any risk of their surrounding area, that is to say the containers 3 surrounding them, being thawed

by the selective heating process. That arrangement, by virtue of suitable temperature adjustment and regulation of the temperature-control elements 2, allows the temperature of one or more containers 3 to be controlled selectively at a temperature between about 20°C and about 70°C above the temperature of the remaining containers.

In addition to the device described above, in which to control the temperature of individual containers 3 of a container array 30, that array 30 and a temperature-control element 2 had to be positioned relative to one another in a plurality of linear relative movements, it is also possible to use devices of different construction for selectively controlling the temperature of individual containers 3. Figures 6 to 8 show, merely by way of example, three such devices in a roughly diagrammatic form. Those devices correspond in their basic principle to the temperature-control device known from WO 99/16549, which has an earlier priority but was post-published, but can all be equipped according to the invention with additional cooling zones 16, as described above.

In the embodiment according to Fig. 6, Peltier elements 18 are mounted on each holder 4 so as to provide a good heat-contact surface to the reaction container in the holder 4. It is known to the person skilled in the art that Peltier elements consist of a cooling side 19 and a heating side 20 which can be appropriately exchanged by reversing the electrical polarity. Such a holding plate 1, as shown in Figure 6, is then able to accommodate reaction containers in an array and to cool them by appropriately switching the Peltier elements. By means of suitable control means, the temperature of individual containers can be controlled selectively by reversing the polarity of the Peltier elements. In order to be able to establish exact temperature control, it may accordingly be advantageous to supply or remove heat by means of a further plate 17 in Fig. 6, in order thus to achieve simpler and more precise regulation. For that purpose, a temperature-controllable plate 17 as in Fig. 6 (here, for example, on the base) may be advantageous according to the invention. When used for thawing, the plate 17 should be cooled in order to dissipate the heat used for heating.

A further embodiment according to the invention is shown in Fig. 7. Here individual holders 1 having depressions 4 for holding containers 3 are coupled to a Peltier element 18 at the base. The individual holders are separated from one another by a heat-insulating layer 28. For dissipating heat there is mounted on the base a suitable air channel 21 which serves for cooling the Peltier elements 18 by means of a fan 12. In a manner comparable to that in Figure 6, selective temperature-control or heating can be achieved by reversing the polarity of the Peltier elements 18.

According to Fig. 8, the area surrounding the holders 4 for the containers 3 is supplied with cooling or heating fluid. The coolable holders 1 in question are separated from one another individually by means of an insulating layer 28 and each have bores 22 which can be supplied with cooling or heating fluid. These can be suitable cooling coils or, when the coolable holding plate is made of metallic materials, also suitable bores with connections. The individual connections can all be controlled using a suitable valve system so that individual holders can be cooled or heated selectively.

The devices according to Fig. 6 to 8 are particularly suitable for nucleic acid hybridisation in a plurality of containers with different sequences and thus different hybridisation temperatures. It should again be expressly emphasised that those three embodiments are merely possible configurations according to the invention.

Claims

1. Device (40) for selectively controlling the temperature of at least one container (3) of an array (30) having a plurality of such containers (3),

   the device comprising a holding plate (1) for the container array (30) which serves to maintain the container array (30) at a predetermined temperature,

   the holding plate (1) having at least one through-opening (5) by means of which at least one temperature-control element (2) can be brought into heat-exchange contact with a container (3) the temperature of which is to be controlled, and

   the container array (30) and the at least one temperature-control element (2) being movable relative to one another.

2. Device according to claim 1,
   characterised in that the holding plate (1) comprises a plurality of longitudinal channels (4) in each of which a plurality of containers (3) of the container array (30) can be accommodated.

3. Device according to claim 2,
   characterised in that each of the longitudinal channels (4) has at least one through-opening (5), preferably in the region approximately of its longitudinal centre.

4. Device according to claim 3,
   characterised in that a drive device (Y) is provided for positioning the container array (30) relative to the least one through-opening (5), preferably by displacement of the container array (30) in the longitudinal channels.

5. Device according to claim 3 or 4,
   characterised in that a drive device (X, Z) is provided for positioning the at least one temperature-control element (2) relative to the at least one through-opening, preferably by displacement of the temperature-control

element in a first direction (X) orthogonal to the longitudinal channels (4) but substantially parallel to the plane of the holding plate (1) and in a second direction (Z) substantially orthogonal to the plane of the holding plate (1).

6. Device according to any one of the preceding claims, **characterised in that** a counterhold device (15), for example a counter-hold plate (15), is provided, which presses the container array (30) against the holding plate (1).

7. Device according to any one of the preceding claims, **characterised in that** at least one additional cooling zone (16) is provided which dissipates the heat emitted by the temperature-control element (2) and not utilised for controlling the temperature of the container (3).

8. Device (40) for selectively controlling the temperature of at least one container (3) of an array (30) having a plurality of such containers (3) by means of at least one temperature-control element (2), if desired in accordance with any one of the preceding claims,

the device comprising a holding plate (1) for the container array (30) which serves to maintain the container array (30) at a predetermined temperature, and

at least one additional cooling zone (16) being provided which dissipates the heat emitted by the temperature-control element (2) and not utilised for controlling the temperature of the container (3).

9. Device according to claim 7 or 8, **characterised in that** the additional cooling zone (16) comprises a ring element made of material having good thermal conductivity which is thermally coupled to a cooling device.

10. Device according to any one of claims 7 to 9, **characterised in that** when a plurality of additional cooling zones (16) is

provided, at least some of those cooling zones (16) are thermally coupled to a common cooling device.

11. Device according to any one of claims 7 to 10, **characterised in that** the temperature difference between the holding plate (1) and the additional cooling zone (16) is between about 5°C and about 50°C, preferably between about 20°C and about 30°C.

12. Device according to any one of the preceding claims, **characterised in that** the temperature-control element (2) comprises at least one Peltier element.

13. Device according to any one of the preceding claims, **characterised in that** the temperature-control element (2) comprises at least one heating coil.

14. Device according to any one of the preceding claims, **characterised in that** the temperature-control element (2) is surrounded by a heat-insulating layer (8).

15. Device according to any one of the preceding claims, **characterised in that** a plurality of temperature-control elements (2) is provided, the temperature of which can preferably be controlled independently of one another.

16. Device according to any one of the preceding claims, **characterised in that** the container array (30) comprises an array plate (11) on which the plurality of containers (3) is integrally formed.

17. Device according to any one of the preceding claims, **characterised in that** the spacing (A) between adjacent containers (3) is between about 1 mm and about 10 mm, preferably between about 2 mm and about 5 mm.

18. Device according to any one of the preceding claims, **characterised in that** the spacing (B) from the array plate (1) to the base of the containers (3) is between about 1 mm and about 100 mm, preferably between about 5 mm and about 35 mm.

19. Device according to any one of the preceding claims, **characterised in that** the wall thickness of the containers (3) is between about 0.2 mm and about 0.5 mm, preferably between about 0.2 mm and about 0.3 mm.

20. Device according to any one of the preceding claims, **characterised in that** the thickness of the array plate (11) is between about 0.5 mm and about 5 mm, preferably between about 2 mm and about 4 mm.

21. Use of a device according to any one of the preceding claims for screening, preferably high-throughput-screening, for example in molecular biology, such as, for example, in nucleic acid hybridisation.